Studies on the Production of Xylose from Water Hyacinth

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Abstract

Water hyacinth is a fast growing noxious weed in the tropical countries. A significant amount of ethanol can be produced from sugar obtained by saccharification of water hyacinth substrate. The present work emphasizes the studies on sulphuric acid pretreatment of water hyacinth for production of xylose. The xylose yield has been studied with the parametric variation of soaking time, treatment time, treatment temperature and the concentration of sulphuric acid. The maximum value of xylose yield obtained is around 70.2 mg/g of dry water hyacinth, when hydrolyzed with 5% sulphuric acid at a temperature of 130 °C, soaking time of 45 min and treatment time of 10 min. The yield corresponds to the initial loading of 2.5%. The yield drops drastically on increasing the temperature above this point which may be due to the degradation of xylose. A correlation of xylose yield with soaking time, treatment time, treatment temperature and acid concentration has also been determined during this study.

Keywords

Water Hyacinth; Sulphuric Acid Pretreatment; Xylose; Correlation; Acid Concentration

Introduction

Global depletion of energy supply due to the continuing over-utilization is being a major problem of the present and future world community. It is estimated that the fossil fuels will be running out by the next few decades [Bentley, 2002 ; Cavallo, 2002] and therefore attention has currently been dedicated to the conversion of biomass into fuel ethanol. Domestic production and use of ethanol as an alternative fuel can decrease dependence on foreign oil reduce trade deficits; create jobs in rural areas, reduce air pollution, and reduce global climate change due to carbon dioxide buildup. Over the last few years, shortage of petroleum and the increase in its price which lead to serious energy crisis has created another way of utilization of alcohol as an energy source. Since ethyl alcohol having a net calorific value of 7100 cal/ g (approx) generates smokeless and odorless products during combustion, it offers a possibility for its use in small stoves in certain situations. It can be used for the lighting purpose also in rural areas. Bioethanol blended in different proportions with gasoline or diesel is already in use as a fuel in automobiles. Ethanol, unlike gasoline, is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NO_2 emissions from combustion.

Ethanol can be made synthetically from petroleum or by microbial conversion of biomass materials through saccharification and fermentation [Masende et al., 1999]. The lignocelluloses are by far, the earth's most prevalent renewable organic materials available for microbial or other conversions. Production of ethanol from food materials e.g. sugarcane bagasse, corn fiber, rice straw, cassava waste etc., has received widespread interest due to their availability, abundance and relatively low cost [Gonzalez et al., 1986 ; Pessoa et al., 2007]. Ethanol with a chemical formula C_2H_5OH is a key component of alcoholic beverages. It also has other common names such as alcohol, ethyl alcohol, grain alcohol, cane alcohol, wine spirit and cologne spirit. It is a colorless, transparent, neutral, volatile, flammable, oxygenated liquid hydrocarbon, which has pungent odour and a sharp burning taste. Because of its relatively high affinity for both water and organic compounds ethanol has found considerable industrial applications.

Water Hyacinth (Eichhornia crassipes) is a monocotyledonous freshwater aquatic plant, belonging to the family Pontederiaceae, related to the lily family (Liliaceae) and is a native of Brazil and Equador region. It is a well known ornamental plant found in water gardens and aquariums, bears beautiful blue to lilac colored flowers along

with their round to oblong curved leaves and waxy coated petioles. It grows from a few centimeters to about a meter in height. The stem and leaves contain air filled sacs, which help them to stay afloat in water. In the developing world, it is used in traditional medicine and even used to remove toxic elements from polluted water bodies. They reproduce both asexually through stolons and sexually through seeds, which remain viable for up to 20 years and therefore are difficult to control. Thus, it is also considered as a noxious weed in many parts of the world as it grows very fast and depletes nutrient and oxygen rapidly from water bodies, adversely affecting flora and fauna. Moreover due to high evapo-transpiration it adds to water crisis all over the places where it grows. It is reported that under favourable conditions water hyacinth can achieve a growth rate of 17.5 metric tons per hectare per day [Mahmood et al., 2009].

The possibility of converting water hyacinth to biogas or fuel ethanol is currently established in a number of developing countries, mainly in India [Sharma et al., 2002]. Production of food, fuels and chemicals from materials considered as "waste" constitutes a valuable service in the self-sustaining society we might envision for the future. Lignocellulosic hydrolysates, however, contain substances that inhibit microbial fermentation to desirable products. The yeast Saccharomyces cerevisiae is relatively resistant to inhibitors [Lindén and Hahn-Hägerdal., 1996] but detoxification may still be necessary in order to reach maximum productivity in the fermentation process. Monoaromatic inhibitors include phenolic compounds formed during the pretreatment process by de-gradation of lignin, aromatic plant polymers synthesized from phenylpropanoid precursors. Phenolic compounds also occur in wood as extractives [Rowe et al., 1989; Sjöström., 1993]. In addition, a variety of phenolic compounds have been reported to form in acidic aqueous solutions of carbohydrates (including wood polysaccharide components such as D-glucose, D-xylose, L-arabinose, D-glucuronic acid, and D-galacturonic acid) at elevated temperatures [Popoff et al., 1972; Popoff et al., 1976]. White-rot fungi [Eriksson et al., 1990] are known for their ability to degrade lignins. Hardwood and softwood in general contain 20%-25% and 26%-32% lignin respectively [Sjöström, ., 1993] As white-rot fungi degrade large amounts of wood aromatics, the enzymes they secrete for this purpose should be of interest for investigations concerning the detoxification of aromatics derived from wood. One of the best-studied white-rot fungi is the Basidiomycete (recently renamed Basidiomycota), Trametes versicolor, which secretes enzymes such as the phenol oxidase laccase and peroxidase [Jönsson et al., 1995 ; Jönsson et al, 1989] which take part in the transformation of aromatic compounds.

The present study aims at applying a simple and reliable pretreatment process with diluted acid hydrolysis for the conversion of water hyacinth to xylose. The water hyacinth used in this study has a composition of 40.71% cellulose, 23.7% hemicellulose, 15.42% lignin as determined in the laboratory. The initial loading used in the study was 2.5%. Estimation of xylose which is a major fermentable sugar was performed by spectrophotometric determinations. Moreover, different parameters like treatment temperature, treatment time, concentration of the medium, soaking time of the dried biomass have been considered and detailed studies were carried out with water hyacinth to establish the variation of xylose yield.

Materials and Methods

Preparation of Water Hyacinth

Fresh water hyacinth with long stem was collected from a natural pond. The water hyacinth was thoroughly washed several times with tap water to remove adhering dirt, chopped into small pieces of size 1-2 cm (approx), and further grounded to even smaller particles of size 2 mm (approx), and finally dried in a hot air oven at 106 °C for 6 hour. The dried material was stored at room temperature until used. The dried water hyacinth was studied under a Magnus Microscope Model MLX-B with LED compound microscope for its cell structure.

Preparation of Hemicellulose Acid Hydrolysate

A sample (0.5 g) of dried water hyacinth was mixed with 1%, 2%, 3%, 4%, 5% concentration of sulfuric acid to a final volume of 20 ml. The mixtures were soaked for 15 min., 30 min., 45 min. and 60 min. The acid hydrolysis reactions were carried out in the temperature range of 40 °C to 150 °C for a treatment time range of 2-10 min. after which the hydrolysate was cooled down to room temperature. The hydrolysate was filtered using what man paper no. 1

to remove the unhydrolysed material. The filtrate was collected and subjected to analyze the xylose.

Determination of Xylose Content by Phloroglucinol Assay

Xylose content was determined using the Phloroglucinol assay [Eberts et al., 1979 ; Johnson et al., 1984] with the hydrolysate obtained from acid hydrolysis. The coloring reagent mixture was heated in water bath and rapidly cooled to room temperature before measuring in a THERMO UV1 100 Double Beam scanning Spectrophotometer at 570 nm.

Results and discussion

Successful bioconversion of lignocelluloses from locally available water hyacinth to xylose production has been achieved by using acid hydrolysis. Hydrolysis of water hyacinth by dilute acid yields mixture of sugars with xylose as a major component (60% approx) [Malik et al., 2007]. It has been noticed that an increase in acid concentration from 1% to 5% leads to twofold increase in the xylose yield. The maximum xylose yield from dried water hyacinth was found up to 70.2 mg/ g in the acid hydrolysate. The results are in good agreements with previous reports on the acid treatment of hemicellulose [Pessoa et al.,1997 ; Roberto et al 1994 ; Elander. et al. 1995] generation of furfural, a by-product of xylose degradation [Ackerson et al., 1981] as a consequence of acid hydrolysis is to be kept in mind during the pretreatment process. The rate of degradation depends on temperature and concentration of sulfuric acid [Gonzalez et al.,1986].

The cell structure of water hyacinth during the pretreatment by sulfuric acid with different parametric variants is studied which is depicted through the photographs of compound microscope as shown in Fig. 1a and SEM pictures in Fig.1b.

The composition of the water hyacinth, subjected to various experimental conditions, used in the experiment was analysed in the laboratory and is shown in Table 1.

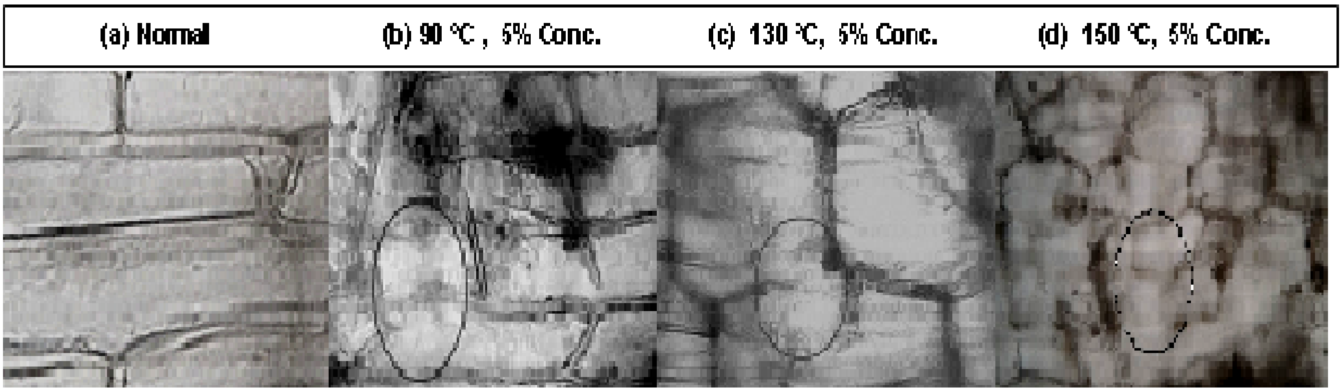

Fig. 1A PARTIAL DEGRADATION OF CELL WALL DURING THE PRETREATMENT PROCESS

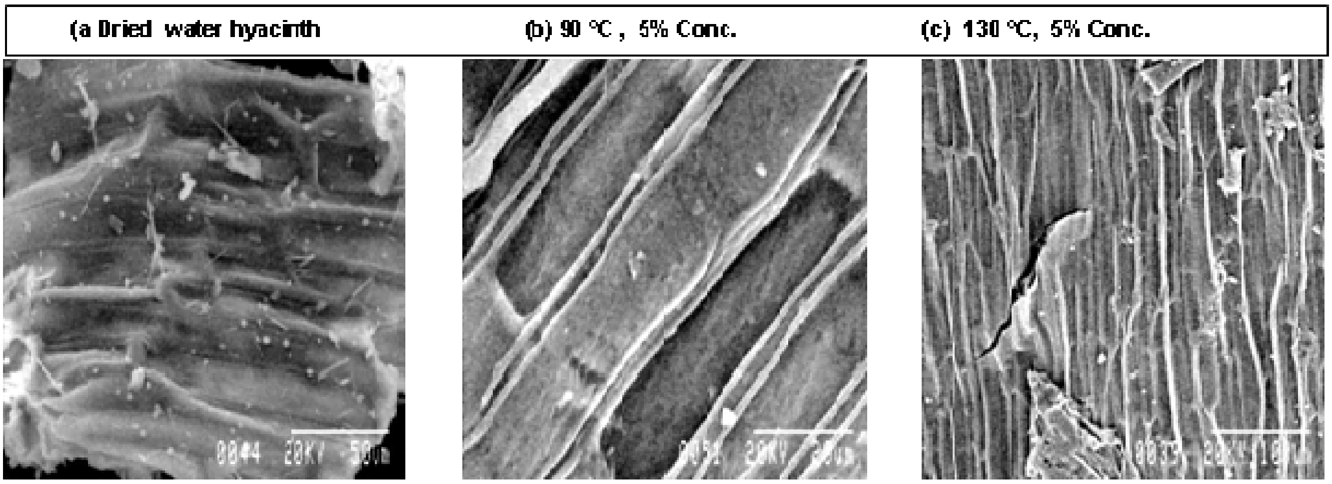

Fig. 1b SEM PICTURES OF PARTIAL DEGRADATION OF LIGNIN DURING THE PRETREATMENT PROCESS

TABLE 1 ANALYSIS OF WATER HYACINTH FOR LIGNIN AND HEMICELLULOSE

Raw Material	Hemicellulose (%)	Lignin (%)	Experimental Conditions
Fresh	23.7	15.42	Room temperature, untreated
Dried	16.6	15.67	106 °C, 6 h, untreated
90 °C	13.33	14.87	C - 5%, t_t – 10 min, t_s – 60 min
110 °C	11.6	14.58	C - 5%, t_t – 10 min, t_s – 60 min
130 °C	9.8	14.34	C - 5%, t_t – 10 min, t_s – 60 min
150 °C	7.4	14.21	C - 5%, t_t – 10 min, t_s – 60 min

From the analysis done with water hyacinth it has been found that the hemicellulose percentage decreases with increase in temperature during hydrolysis with 5% sulfuric acid and a treatment time of 10 min. as shown in Fig. 2.

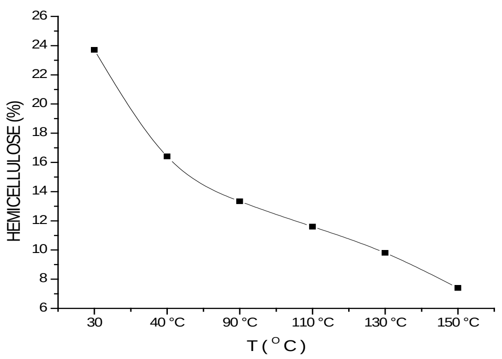

Fig. 2 VARIATION OF HEMICELLULOSE YIELD WITH TEMPERATU

However the change in lignin composition was not significant under the same conditions. The effects of varying soaking time, treatment time, treatment temperature and concentration of sulphuric acid on the xylose yield have been investigated. The xylose yield becomes doubled for a change of treatment time from 2 min. to 10 min. This is shown in Fig. 3.

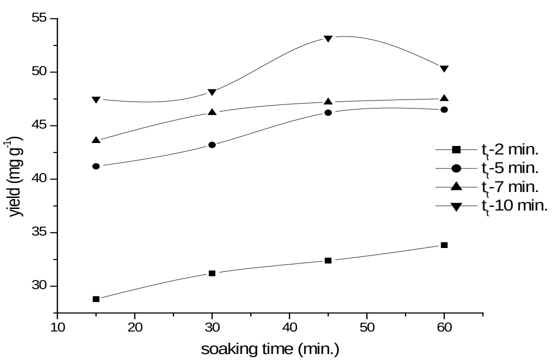

Fig. 3 EFFECTS OF SOAKING TIME AND TREATMENT TIME ON THE XYLOSE YIELD

Maximum yield was obtained with treatment time of 10 min and soaking time of 45 min.

A sharp increase in yield of xylose was recorded at higher temperature range from 40 °C onwards. The highest yield of xylose was found at 130 °C while at more elevated temperatures the yield decreased.

This may be attributed to the degradation of xylose and production of additional compounds. Fig. 4 shows that with the variation of temperature from 40 °C to 150 °C the yield of xylose increased twice keeping the soaking time and concentration constant at 60 min. and 5% respectively.

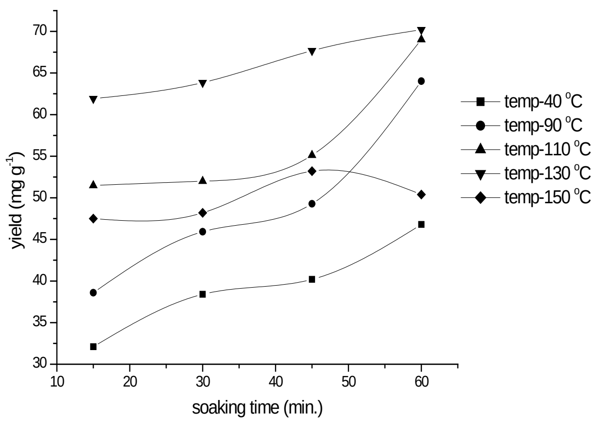

Fig. 4 CHANGE OF XYLOSE PRODUCTION WITH THE SOAKING TIME AND TEMPERATURE

The production of xylose increases with the increase in concentration of the solution. When varying the concentration from 1%, to 5% the yield of xylose increased one and half times keeping the soaking time and temperature constant at 10 min. and 150 °C respectively. However the maximum yield was obtained at 3% concentration and further increase in concentration at such elevated temperature of 150 °C resulted in the loss of xylose yield which may be due to the degradation of xylose as shown in Fig. 5.

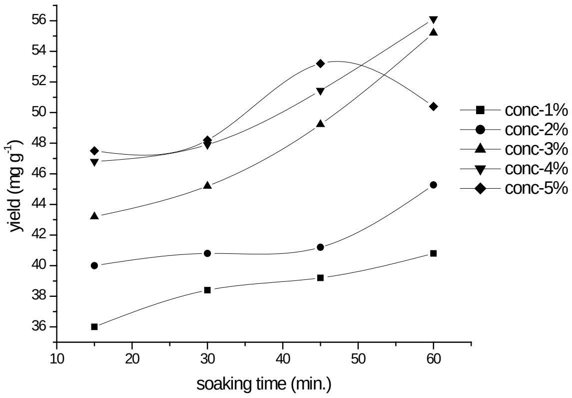

FIG. 5 XYLOSE YIELD WITH CHANGE IN SOAKING TIME AND CONCENTRATION OF SULPHURIC ACID

When varying the temperature from 40 °C to 150 °C, it was found that the maximum increase in xylose was 69.6 mg/g at 130 °C at a soaking time of 45 min. With the further increase in the temperature and soaking time, the xylose yield falls back. This may be due to the degradation of xylose at higher temperature into furfural [Carlo et al., 2005]

While studying the effects of concentration it has been observed that at 3% concentration of acid in the temperature range of 150 °C the yield of xylose is maximum (67.68 mg/g). With a further increase in temperature the xylose yield drops indicating the degradation of the xylose to its further derivatives [Parveen et al. ,2009]. The yield of xylose increases when treatment time is varied from 2 min to 10 min. keeping concentration and temperature constant at 5% and 150 °C respectively. Maximum yield was obtained with treatment time of 10 min [Sun et al ., 2002].

Correlation

The yield of xylose when hydrolysing water hyacinth with sulphuric acid has been studied against treatment temperature, concentration of acid, treatment time and soaking time. The experimental data obtained have been correlated by the following expression using multiple regression technique and found to account within ± 12% of all observations as shown in Fig. 6.

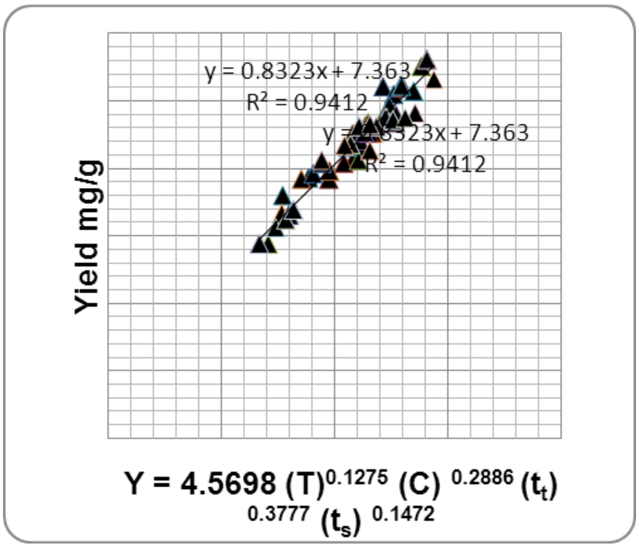

Fig. 6 Correlation of xylose yield with process variables

$$Y = 4.5698(T)^{0.1275}(C)^{0.2886}(t_t)^{0.3777}(t_s)^{0.1472}$$

The goodness of the above analysis is justified by the coefficient of multiple determination / correlation co-efficient which has been computed as 0.941within the range of parameters used in this study.

Conclusion

The present investigation on acid hydrolysis of water hyacinth has been carried out in the temperature range of 40 °C to 150 °C. It has been observed that at an elevated temperature in the range of 110 °C to 150 °C and low concentration of acid (1% - 5%) the dilute acid pretreatment achieved high reaction rates and significantly improved the cellulose hydrolysis. At the temperature of 150 °C and acid concentration of 1% with treatment time of 2 min the xylose yield recorded was 14.4 mg/g, while at the same temperature and using acid concentration of 5% and treatment time of 10 min., the yield significantly increased to 50.4 mg/g. As recorded in the present study, it has been noticed that with dilute acid hydrolysis processes using less severe conditions higher xylose conversion of 70.2 mg/g can be achieved at 130 °C, 5% acid concentration and 10 min treatment time than at elevated temperatures. High xylan to xylose conversion is necessary to achieve favourable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials [Hinman, et al., 1992]. As found in the experimental study, 46% of hemicellulose removal was possible at the temperature of at 90 °C while 69% of the total hemicellulose was converted to xylose at an elevated temperature of at 150 °C. Thus high degree of hemicellulose removal is possible by dilute acid pretreatment, whereas the pretreatment did not have any significant effect on lignin at different ranges of temperature. Only 3.5% lignin removal was possible at 90 °C, while even at an elevated temperature of 150 °C the lignin removal was only 7.8% indicating no significant changes with the increase in temperature.

In the experiment which have been carried out for a varied range of parameters it was found that the maximum yield of xylose was 70.2 mg/g at 130 °C with a treatment time of 10 min. and soaking time of 45 min. When increasing the temperature further to 150 °C the yields drops drastically to 50.4 mg/g which may be due to degradation of xylose to furfural and other derivatives at higher temperature.

A non linear regression model has been developed from the experimental data to predict the xylose yield with the changes in acid concentration, soaking time, and treatment time and treatment temperature. It has been observed that all the experimental data

fall within ± 12 % of the predicted values through this correlation. The value of correlation co-efficient is 0.941 which is very much encouraging.

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Nomenclature

T- Treatment temperature, ^oC

C- Concentration, %

t_t- treatment time, min.

t_s- Soaking time, min.

Y- Yield, mg/g

R^2- Coefficient of determination

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